SENESCENT CHANGES IN THE KIDNEY OF THE YELLOW MUD TURTLE *KINOSTERNON FLAVESCENS*

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by

Tanya M. Williams

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An abstract of a thesis by

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Current literature suggests that reptiles do not undergo senescence. This study addresses age-related changes that occur in the kidneys of reptiles and compares them to senescent changes in mammalian kidneys. Kidney sections from a wild population of turtles ranging in age from 6 to 35 years were examined histologically by light and electron microscope. Kidney tubules of the older turtles showed a statistically significant greater deposition of a pigment believed to be lipofuscin or ceroid than did young turtles. Glomeruli in the oldest turtles had significantly fewer glomerular arterioles, and a significant increase in connective tissue in the glomerular tuft. Electron microscope studies indicated that glomeruli became smooth and simplified with advanced age. Foot processes lost their regular arrangement and became wider. Pedicels became irregular or degenerate. Fine structures of reptilian kidney appeared to be simpler and more organized than mammal kidney, but age-related changes were similar.

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INTRODUCTION

Senescence is the process of age-related deterioration exclusive of infectious disease (Christiansen and Gryzbowski 1993). Although the pathology of senescence of mammals, especially humans, has been well documented (Anderson 1971), senescence is not as clearly described in reptiles. It has been suggested that reptiles undergo three types of senescence: rapid, gradual, and slow or negligible, depending on the order to which the organism belongs (Patnaik 1994). Turtles have been attributed with slow or negligible senescence (Patnaik 1994), and it has even been postulated that they do not undergo an aging process at all (Gibbons 1987, 1992).

Turtles seldom appear to die of old age. Common causes of death seem to be predators, severe environmental conditions, and accidental death from automobiles and other causes. Turtle longevity can be attributed to several factors. Because turtles are ectotherms, they have a slow metabolic rate at low temperatures. Sacher (1978) suggests that senescence is related to metabolic rates, so turtles could be younger physiologically than endotherms of the same chronological age (Gibbons 1987). In addition, turtles have a long period of maturation compared to other animals. A long life span would be necessary to compensate for the slow maturation and resulting delayed reproduction observed in turtles. Longevity also helps the turtle to compensate for the high number of nests and hatchlings that fall prey to predators, since turtles are reported to produce successive clutches for a lifetime, ensuring reproductive success (Gibbons 1987, Patnaik 1994). There may be longevity benefits from the protection provided by the shell (Gibbons 1987).

A few studies show some evidence of senescence in other reptiles. Age-related decline in metabolic rate and growth rate has been observed in lizards, as well as age pigmentation and cell loss in major organs (Patnaik 1994). None of these changes has been observed in turtles to date, although one unpublished study reports an increase in the

percentage of connective tissue in the arterial walls of *Kinosternon flavescens* (turtles) (Lyons 1995). Among reptiles, chelonians are reported to have the greatest longevities, followed by crocodilians and *Sphenodon* (Castanet 1994).

The present study examines histologic changes that take place in the kidney and heart of the Yellow Mud Turtle, *Kinosternon flavescens*, a representative of the family Kinosternidae from Iowa and Nebraska. To date, no evidence has been published showing that the senescence process occurs in turtles of any family.

Numerous morphologic age-related changes have been reported in mammalian kidneys, notably in the renal blood vessels, glomeruli, tubules, and interstitium. Histologic studies have shown a decrease in the number of tubular cells per unit area, glomerular tufts, and cells per glomerular tuft; and an increase in the size of Malphigian corpuscles, the glomerular tuft, glomerular cell nuclei, and the nuclei of tubular cells (Goyal 1982, Heudes et al. 1994). A focal thickening of the basement membranes of glomeruli, Bowman's capsule, and convoluted tubules has also been observed (Darmady et al. 1973, Goyal 1982, Heudes et al. 1994), as well as glomerular sclerosis, and diverticuli in the distal convoluted tubules (Darmady et al. 1973, Goyal 1982, Heudes et al. 1994, Radke 1994).

Several studies of mammalian kidneys report age-related loss of functional glomeruli. It has been estimated that by age 70, humans have 2/3 to 1/2 the number of glomeruli of a normal middle-age adult (Darmady et al. 1973, Heudes et al. 1994, Moore 1931), and the kidney itself loses about one fifth of its size (Christiansen and Gryzbowski 1993). Darmady et al. (1994) reports an increase in the amount of connective tissue in the blood vessels of the kidney. It has been suggested that this vascular disturbance may be the cause for the loss of functional glomeruli (Oliver 1952).

Several changes occur in the human heart with age. Histological changes include interstitial fibrosis, lipofuscin accumulation, collagen cross linking, and amyloid

accumulation (Christiansen and Gryzbowski 1983, Lie and Hammond 1988, Waller 1988). Waller (1988) and Lie and Hammond (1988) also report basophilic degeneration and increased subepicardial fat.

Many of the same age-related pathologies seen in mammals have also been noted in fish. Histologically, evidence of aging includes loss of muscle fibers in the heart, degenerative changes in the nephrons of the kidney, and accumulations of lipofuscin. An increase in collagen cross-linking was also noted (Patnaik et al. 1994).

MATERIALS AND METHODS

Animals

Specimens of *Kinosternon flavescens* used in this study were obtained from the Big Sand Mound population in Muscatine Co., Iowa, under Scientific Collecting Permit #SC2559501 issued to Dr. James L. Christiansen of Drake University. Others were provided by Dr. John B. Iverson from Cherry and Dundee Counties, Nebraska (Fig. 1). After capture, the turtles were kept for a brief period in glass aquaria at 23° C ±3° and fed a diet of fish by Dr. Christiansen. These specimens were from monitored wild populations and their age estimates ranged from 6 to 35+ years. Ages were estimated from a combination of counts of plastral annulae and recapture records (Cagle 1946). Wear of plastral scutes and slowing or cessation of growth decreases the accuracy ±5 years for 20 year-old turtles, and ±10 years for 30 year-old turtles (Christiansen, per. comm.).

Light Microscope Preparation

Kidneys and hearts for light microscope histological study were fixed in buffered 10% formalin overnight, sometimes longer. The tissue was dehydrated in methanol, saturated with xylene, and inpregnated with paraffin. They were embedded in parrafin blocks, sectioned at 5μm, and mounted.

Sections were stained with both hematoxylin and eosin (H&E) and Mallory's triple stain. Mallory's sections were post-fixed in Zenker's for 12 hours before staining, and were pretreated for 10 minutes in a mixture of phosphotungstic and phosphomolybdic acid to insure that all of the connective tissue was stained. In Mallory's triple stain, the connective tissue (elastin, collagen, etc.) is stained a dark blue while muscle and other tissue is stained shades of pink and red (Humanson 1972).

Kidney slides from turtles DU 2977, DU 2998, and DU 2996 were also stained by other methods to determine the nature of the pigment they contained. These included Oil-

DU catalog number	Estimated age years	Location state	Light microscope study	Electron microscope study
2968	6	Iowa	X	
2997	8	Nebraska	X	
2982	10	Nebraska	X	X
2980	11	Nebraska	X	
2983	14	Nebraska	X	X
2981	17	Nebraska	X	
2953	19	Iowa	X	X
2995	20	Iowa	X	X
2971	24	Iowa	X	X
2998	25	Nebraska	X	X
2970	26	Iowa	X	X
2996	35	Nebraska	X	X

Table 1. Catalog number, age, origin, and studies performed on specimens of *Kinosternon flavescens* examined for age-related renal changes.

Red O for lipids, Prussian Blue for ferric iron, periodic acid-Schiff (PAS) for glycoprotcins, and Jones silver for basement membranes. Unstained slides were examined for autofluorescence. Standard staining and preparation procedures were followed in all cases, and a control slide was stained in all cases to insure that proper staining had occurred.

Light Microscope Examination

Light microscope analysis was based on two representative slides from each specimen, one stained with H&E and one stained with Mallory's triple stain. All quantification was done in a blind procedure, without noting specimen number or age, and all studies were evaluated by at least two people.

H&E stained slides were used to estimate the amount of pigment present in renal tubules, the number of glomeruli demonstrating less than two visible red blood cells as a reflection of the extent of the glomerular increase of non-vascular tissue, and the ratio of distal convoluted tubule lumen to tubular cell height. Mallory's stained sections were used to estimate the amount of connective tissue within the glomerular tuft, the number of glomeruli per unit area, and the average distance between the glomerular tuft and Bowman's space, and the overall extent of renal connective tissue deposition.

The extent of tubules with pigment was determined by examining slides under 400X magnification. Pigment quantity was obtained by using an optical grid. Counts were made of the total number of cells containing pigment on three random, non-overlapping grid areas of each slide. These were averaged to give a mean number of cells containing pigment per unit area per slide.

A measure of the relative amount of vascular tissue in glomeruli was obtained by counts of glomeruli with less than two visible red blood cells. A total of ten different randomly selected glomeruli was counted in the five youngest and the three oldest specimens at a magnification of 250X.

Age extremes were again used to estimate the ratio of distal convoluted tubule lumen diameter to tubule cell height. This was determined by measuring tubule lumen diameter and height of tubular cells with an ocular micrometer at a magnification of 125X. This measurement was taken on five different randomly selected tubules judged to be typical for the slide for each of the three youngest and the three oldest turtles.

Five glomeruli from each of the five youngest and the five oldest turtles were examined to determine extent of glomerular connective tissue. A score of one was recorded for those glomeruli with no visible connective tissue; a two for glomeruli with a small amount of visible connective tissue and a three was recorded for glomeruli that were over half connective tissue. The mean of these scores was used as the amount of glomerular connective tissue present in each turtle.

Number of glomeruli per unit area was determined with an optical grid at a magnification of 125X. Three non-overlapping random counts of glomeruli present in the grid were made in all 12 specimens. Counts included glomeruli that were not entirely in the grid in addition to those contained. Means of these counts were compared as were means of counts examined at 250 X.

The average distance between glomerular tuft and Bowman's capsule was determined by measurements of five randomly selected glomeruli on each slide judged to be typical for the slide. Age extremes were again used to estimate the distance, using the three youngest and the three oldest turtles. The measurement was taken from the tip of the glomerular tuft to the edge of Bowman's capsule. Means of these measurements were used as the distance from glomerular tuft to Bowman's capsule in each turtle.

All heart slides were examined for the presence of pigment and an increase in connective tissue. Extent of pigment was determined by examining whole sections for the presence of any amount of pigment. To determine extent of connective tissue, each section

was given a score of one for small amounts of connective tissue, a two for moderate amounts of connective tissue, and a three for extensive connective tissue.

In addition, all slides were examined for any qualitative differences among the turtles. These included things such as thickness of the basement membrane and the total amount of connective tissue present in the kidney, melanin-containing cells and anything else that seemed unusual.

Light Microscope Data Analysis

The mean amount of pigment present in the tubules and the mean number of glomeruli present in each specimen were compared with the estimated age of the turtles by 2-way ANOVA. Regression lines were plotted to determine the extent of pigment in the tubules related to age, and the mean number of glomeruli related to age. Significance was established if $p<0.05$.

A pooled variance *t*--test was used to test the significance of the following relationships to turtle age: functional glomerular tissue relative to non-vascular glomerular tissue, amount of glomerular connective tissue present, ratio of the tubule lumen diameter and tubular cell height, and amount of space between the tip of the glomerular tuft and Bowman's space.

Scanning Electron Microscopy

Tissues were fixed in 10% formalin in situ, then excised for preparation. Normal scanning electron microscope procedure was followed: dehydration, critical point drying, mounting, and ion sputter coating. Before tissues were mounted, they were fractured by tearing to expose glomeruli. Photographs were taken at varying magnifications on the scanning electron microscope, developed, and printed following normal darkroom procedures.

All electron micrographs were examined for qualitative differences among the specimens. Changes in the size of structures was noted, as well as morphological changes

in the whole glomeruli, the foot processes of the podocytes, and the pedicels of the foot processes.

RESULTS

Examination of renal tubules of 12 turtles with estimated ages ranging from 6 to 35 years showed that turtles over 15 years old typically had numerous tubules with a brownish pigment in the cytoplasm of the epithelial cells. Even though the increase was not linear, a regression line confirmed a significant correlation with age (Figure 1). Sections of a typical young (20 year old) and old (35 year old) turtle showing differences in pigment deposition are shown in Figure 2.

Sections stained with Prussian Blue for ferric iron indicated that the pigment was not hemosiderin. Stain with PAS for glycoproteins and Oil-red O for lipids were positive and faintly positive respectively and the granules fluoresced with a gold-brown autofluorescence. These staining results are indicative of lipofuscin or ceroid (Roberts 1974).

The extent of proliferation of supporting tissue in the glomerular tuft was measured by estimation of the visible glomerular arteriole area through counts of visible erythrocytes. Number of glomeruli with less than 2 erythrocytes (0 or 1) were counted in kidney sections from turtles from the two age extremes. Glomeruli with extensive non-vascular cellular material rarely had more than one or two erythrocytes visible. Healthy glomeruli typical of young turtles and some of the glomeruli of old turtles commonly had 5 to 10 erythrocytes visible. When five turtles less than 15 years old were compared to three turtles greater than 24 years old, the difference was significant ($P = 0.05$) See Table 2. The mass of cells concealing the vascular tissue is also apparent in Figure 3.

Scanning electron microscope (SEM) studies were conducted to further define age-related glomerular changes. Eight turtles with ages ranging from 10 to 35 were examined by SEM and were compared to light microscope slides from the same kidneys as well as to electron micrographs of normal human kidney. Some glomeruli of old turtles often

appeared to be smooth and in some instances were cellular masses with no discernable arterioles or podocytes (Figure 6). This was consistent with the proliferation of supporting material and possible loss of glomerular arterioles observed under light microscopy (Figure 3).

Foot processes of young turtle glomeruli were even and regular in appearance (Figure 7 and 8). The most dramatic change seen in the old turtles was a change in pedicels. Pedicels of some glomeruli were disordered, others appeared to be degenerating, and some lacked pedicels (Figure 7 and 8). Pedicels of glomeruli typical of young turtles have interdigitating neighboring foot processes. Features of glomeruli observed by SEM in turtles of different ages were summarized in Table 6.

Tissues stained with Mallory's trichrome for connective tissue were rated (1-3) with 1 = no connective tissue to 3 for the maximum amount of visible connective (blue) tissue. Again, the increase was not evident in middle aged turtles but when the five youngest are compared with the five oldest turtles, the relationship was statistically significant (Table 4). Photomicrographs of typical glomeruli of turtles aged 8 and 35 years in Figure 4 illustrate the tendency for the increase in connective tissue in the glomeruli with age. It is also apparent from this illustration that the oldest turtles often had more connective tissue throughout the kidney. However, this was less consistent than was glomerular connective tissue and we were unable to adequately quantify it.

A few of the oldest turtles had unusually short cells lining the distal tubules. However, comparison of these tubules with tubules of young turtles showed that tubular epithelial cell height varied greatly among turtles of all ages. Regression plots of a ratio of tubule lumen diameter to tubullar cell height deviated only slightly from the horizontal so no relationship was found,and a pooled variance *t*-test also showed no significant difference (Table 4).

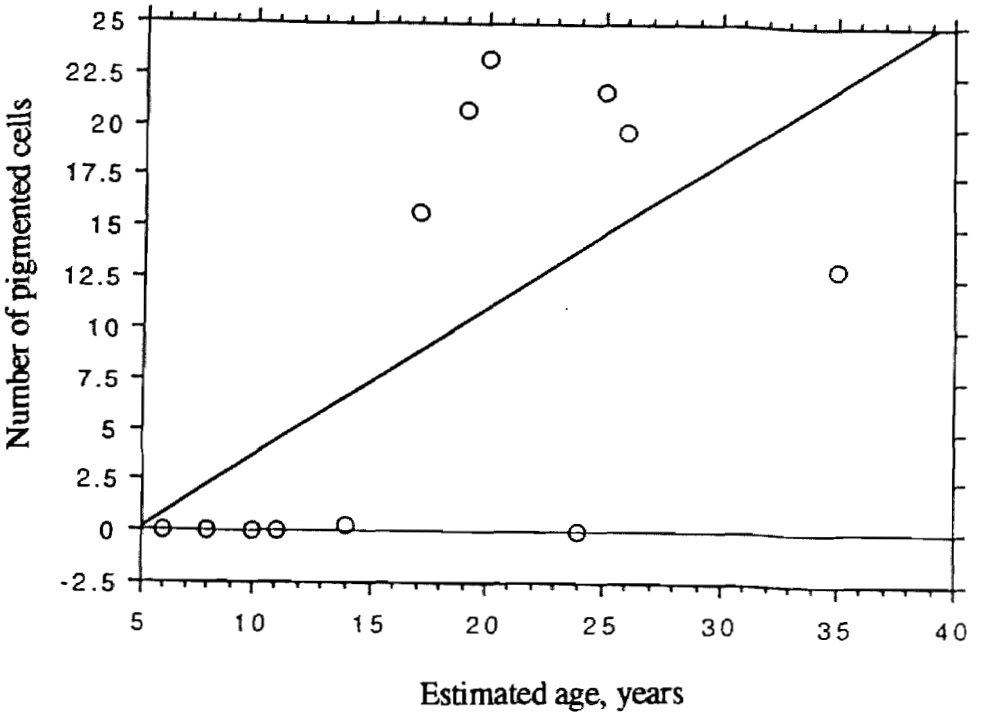
Because the glomerular changes and possible tubular degeneration suggested that glomeruli may be deteriorating with age, we attempted to measure the number of glomeruli per unit area with turtles of different ages. A regression line plotted to show this relationship showed no statistical significance (Figure 5). It could be possible that kidney size declines as nephrons are lost, thereby masking glomerular loss.

An increase in size of the glomerulus with age has been observed in humans (Goyal 1982, Heudes et al. 1994) and this is associated with a decrease in Bowman's space. We were unable to find consistent correlation of these features with turtle age. Table 5 shows a comparison of means of the maximum distance between the glomerulus and the Bowman's capsule. The basement membranes of Bowman's capsule appeared to thicken with an increase in turtle age. It also appeared that there was an overall increase in the total amount of connective tissue present in the kidney as estimated turtle age increased. No statistically significant difference was seen. Because the increase in connective tissue was only seen in the two oldest turtles, the sample size was too small and the variance too large.

No pigment was found in the hearts of any turtles. No consistent differences were seen in the amount of connective tissue present in the heart among old or young specimens.

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	426.741	426.741	5.857	0.0361
Residual	10	728.586	72.859		
Total	11	1155.327			

A



B

Fig. 1. Regression plot of estimated age of *Kinosternon flavescens* and number of pigmented tubular cells per unit area. (P = 0.0361) (A) ANOVA table plotting number of pigmented cells versus turtle estimated age in years. (B) Regression plot. Each dot represents a mean count of the number of pigmented cells in each of three non-overlapping grids for sections of kidney from each turtle. Y = -3.416+.723*X; R^2 = .369

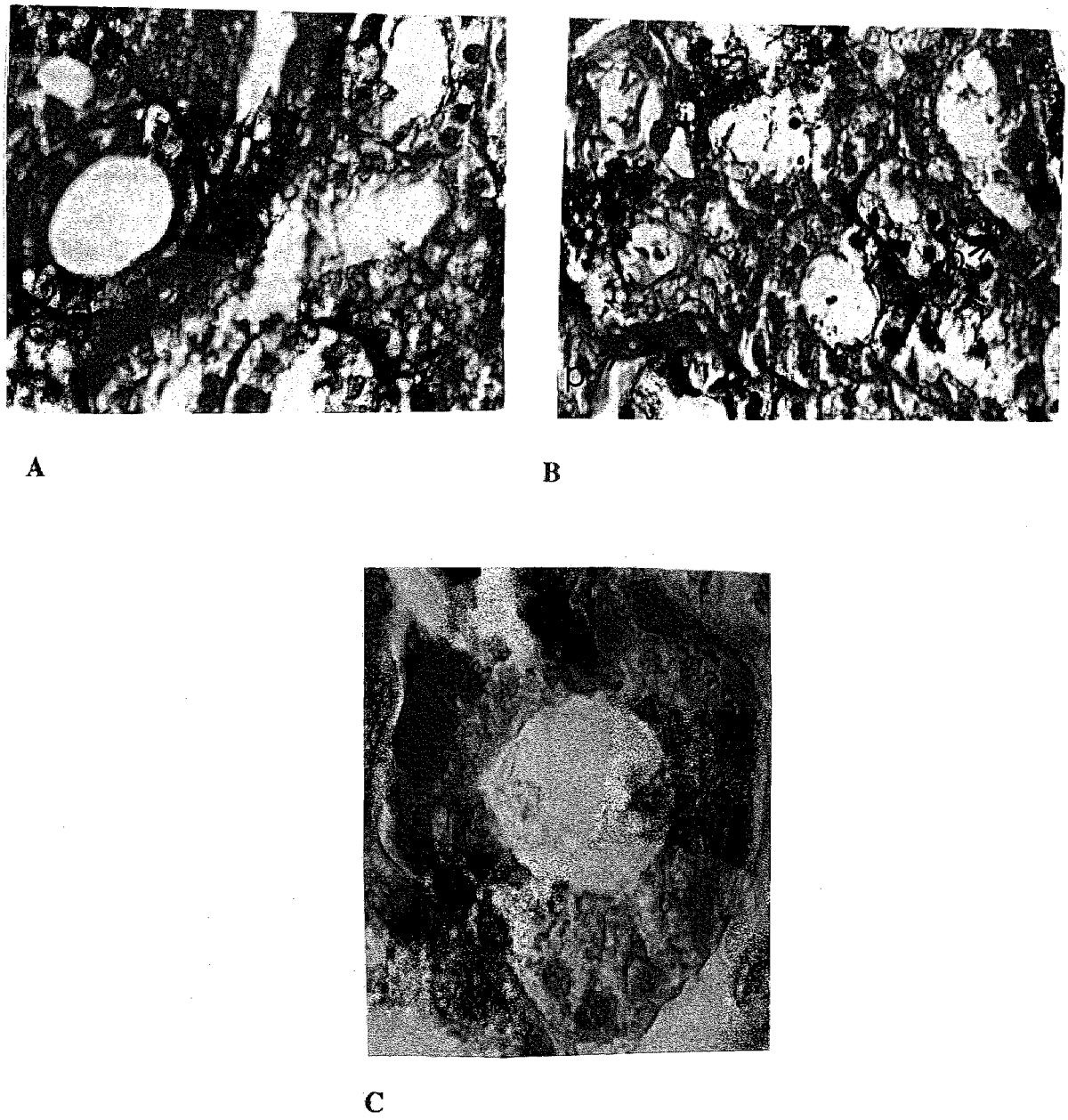
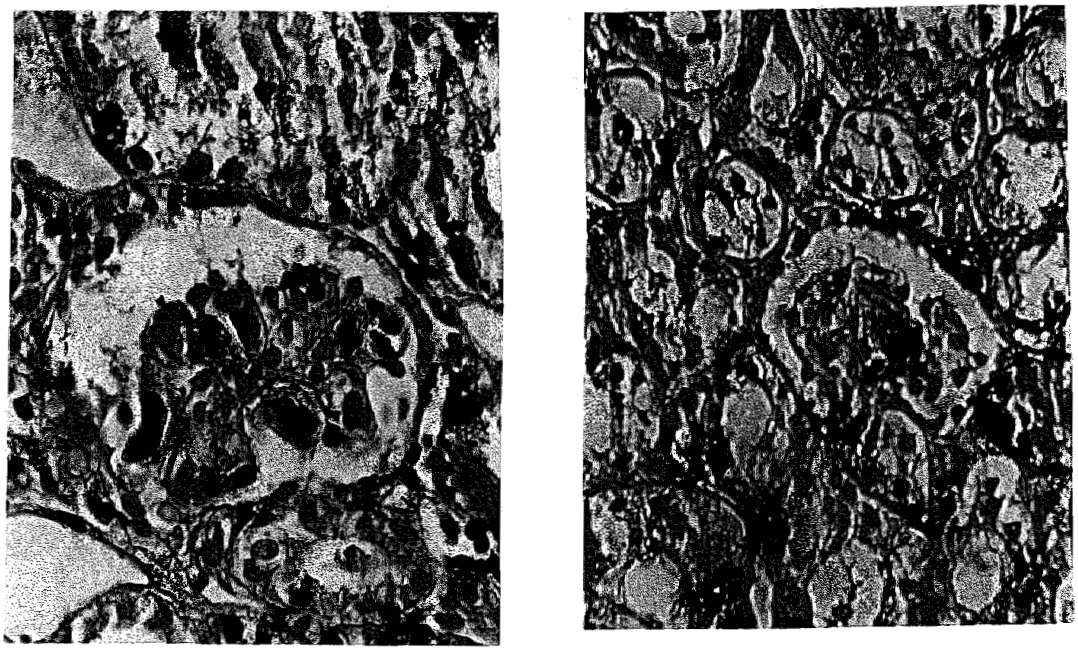


Fig. 2. Light micrographs of renal tubules from *Kinosternon flavescens* 4. (A) Normal tubular epithelium from a 20 year old turtle (DU 2995), 400X. (B) Tubular epithelium from a 35 year old turtle (DU 20996) containing pigment (p), 400X. (C) Single tubule showing pigment granules, 1000X.

	Young (< 15)	Old (> 24)
Number of samples (n)	5	3
Mean	0.000	2.333
t	24.052	
t Critical (P<0.05)	2.132	

Table 2. Results of pooled variance *t*-test. Comparison of the number of glomeruli with only one or zero visible erythrocytes in a young and an old population of *Kinosternon flavescens*.

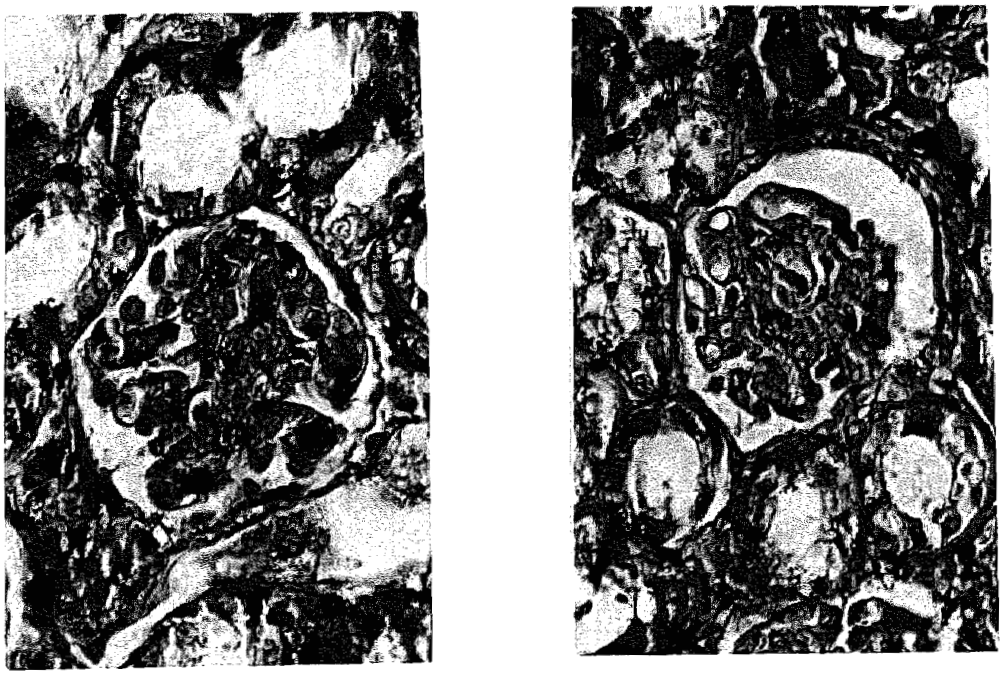


A B

Fig. 3. Light micrographs of glomeruli from kidney of *Kinosternon flavescens*. (A) Glomerulus with nine erythrocytes visible from a 20 year old turtle (DU 2995), 400X. (B) Glomerulus with only one visible erythrocyte from a 26 year old turtle (DU 2970), 250X.

	Young (< 15)	Old (> 19)
Number of samples (n)	5	5
Mean	1.04	1.76
t	3.655	
t Critical (P<0.05)	1.860	

Table 3. Results of pooled variance *t*-test. A comparison of the amount of connective tissue in the glomeruli of a young and an old population of *Kinosternon flavescens*.

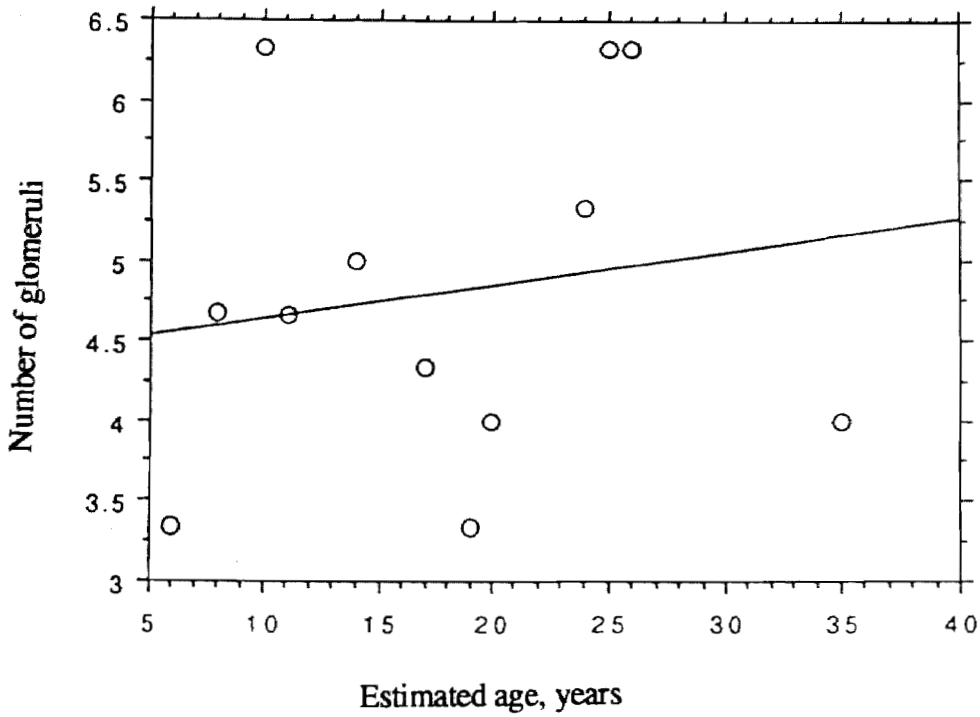


A B

Fig. 4. Light micrographs of glomeruli from kidney of *Kinosternon flavescens*, 400X. (A) Glomerulus form an 8 year old turtle (DU 2997) with no blue connective tissue. (B) Glomerulus with a considerable amount of blue connective tissue within the glomerular tuft from a 35 year old turtle (DU 2996). Both sections were stained with Mallory's triple stain.

	DF	Sum of Squares	Mean Squares	F-Value	P-Value
Regression	1	.346	.346	.269	0.6152
Residual	10	12.857	1.286		
Total	11	13.203			

A



B

Fig. 5. Regression plot of estimated age of *Kinosternon flavescens* and number of glomeruli per unit area. (P = 0.6152) (A) ANOVA table plotting number of glomeruli per unit area versus estimated turtle age in years. (B) Regression plot. Y = 4.435 + .021 * X; R^2 = .026

	Young (< 11)	Old (> 24)
Number of samples (n)	3	3
Mean	2.364	3.532
t	1.481	
t Critical (P<0.05)	2.132	

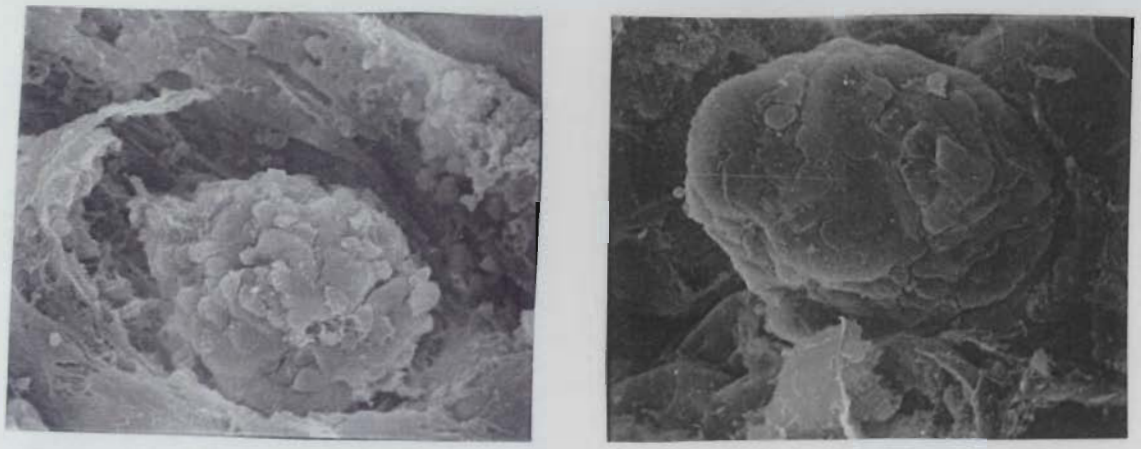
Table 4. Results of pooled variance *t*-test. A comparison of the ratio of tubule lumen diameter to tubular cell height in a young and an old population of *Kinosternon flavescens*.

	Young (<11)	Old (>24)
Number of samples (n)	3	3
Mean	.15	.10
t	1.497	
t Critical (P<0.05)	2.132	

Table 5. Results of pooled variance *t*-test. A comparison of the length from the glomerular tuft to Bowman's capsule in a young and an old population of *Kinosternon flavescens*.

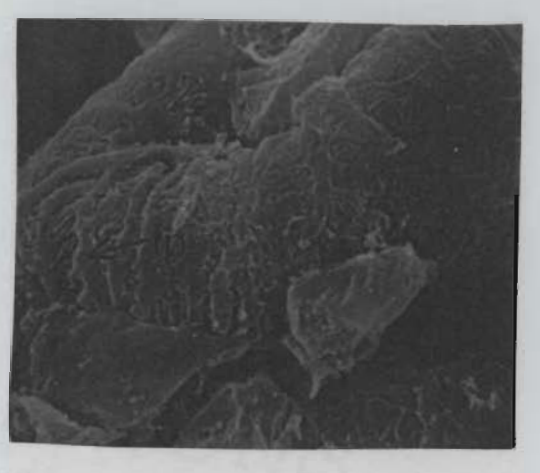
DU catalog no.	Estimated age	Smooth glomeruli	Irregular foot processes	Wide foot processes	Disordered pedicels	Degenerate pedicels	Lack of pedicels
2982	10						
2983	14		X		X		
2953	19					X	
2995	20	X					X
2971	24	X				X	X
2998	25	X		X	X	X	X
2970	26		X	X	X		X
2996	35	X		X	X	X	X

Table 6. Possible age-related changes observed with scanning electron microscopy in glomerular structures of Yellow Mud Turtles. An "X" denotes that the respective specimen demonstrated the characteristic.

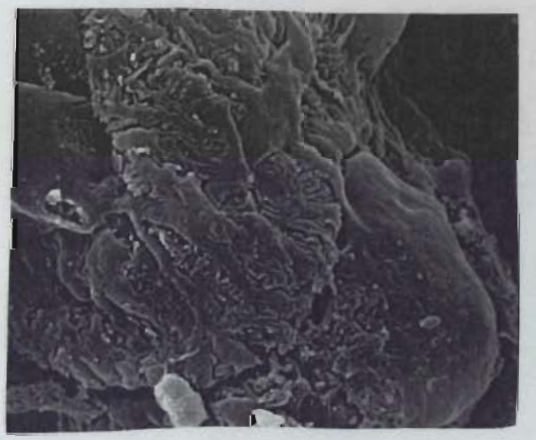


A B

Fig. 6. Scanning electron micrograph of glomerulus of *Kinosternon flavescens*, 650X. (A) Whole glomerulus from a young turtle (DU 2982), age 10 yrs. (B) Whole glomerulus from an old turtle (DU 2995), age 20 yrs. Note the simplification in B. The bulging spheres are podocyte nuclei.



A



B

C

Fig. 7. Scanning electron micrographs of glomerului of *Kinosternon flavescens*, 3500X. (A) Arteriole of a glomerulus showing the regular arrangement of the foot processes (fp) with pedicels interdigitating from a young turtle (DU 2982), age 10 yrs. (B) Arteriole of a glomerulus demonstrating the irregularity of foot processes seen in old turtles (DU 2996), age 35 yrs. (C) Arteriole of a glomerulus showing a lack of pedicels in an old turtle (DU 2998), age 25 yrs.

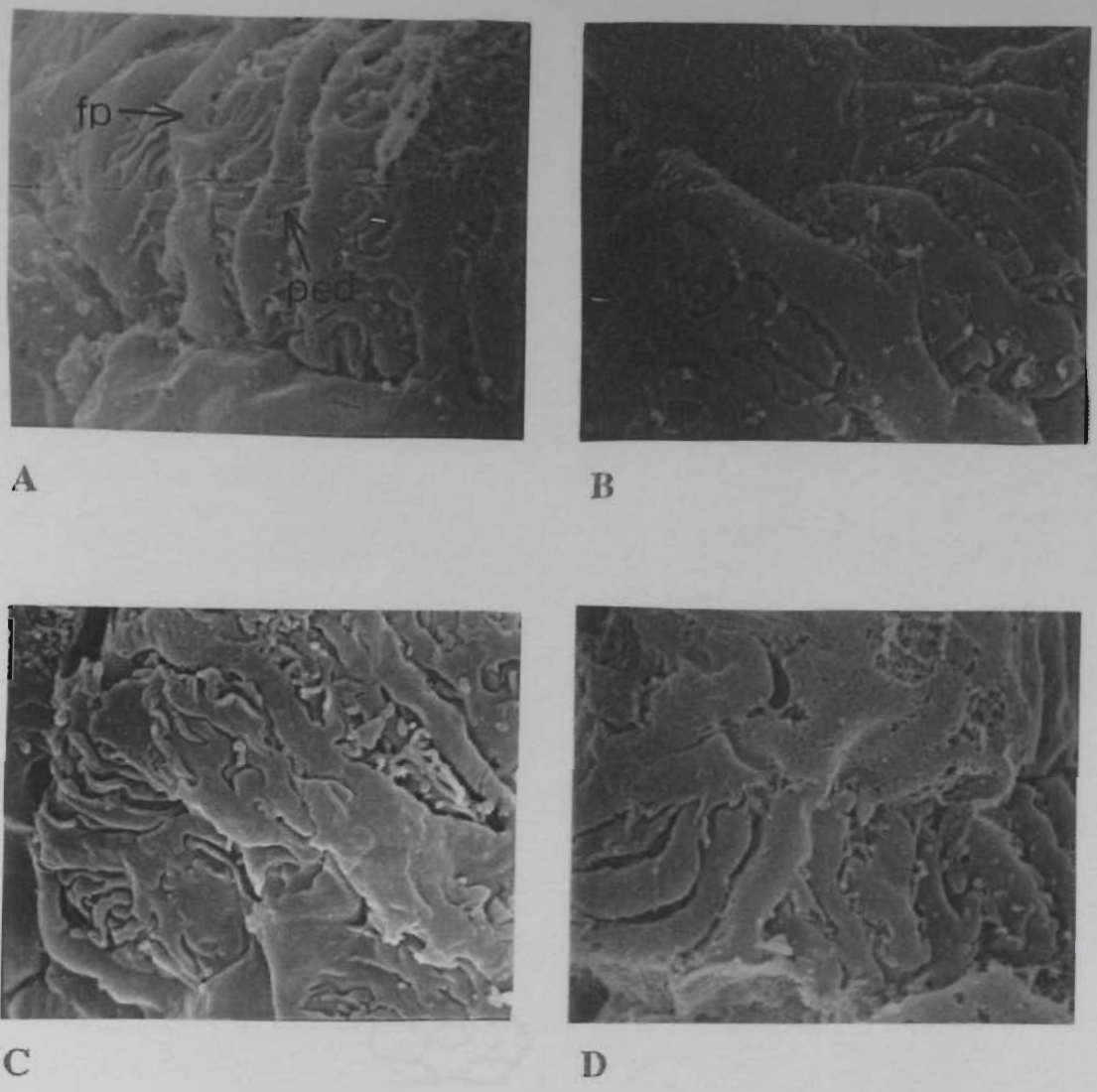


Fig 8. Scanning electron micrographs of glomerular podocytes of *Kinosternon flavescens* kidneys, 8500X. (A) Glomerular arteriole of a young turtle (DU 2982), age 10 yrs of normal appearance. Note the regular arrangement of foot processes (fp) and interdigitation of the pedicels (ped). (B) Arteriole with wide foot processes from an old turtle (DU 2998), age 25 yrs. (C) Arteriole with disordered pedicels from an old turtle (DU 2996), age 35 yrs. (D) Arteriole showing degenerating pedicels from an old turtle (DU 2998), age 25 yrs.

DISCUSSION

It is evident from this study that the kidneys of the Yellow Mud Turtle change with age. This study provides the first report of pigment in tubular epitheluim of reptiles. All results from histochemical tests to determine pigment composition were consistent with lipofuscin or ceroid (Roberts 1974). The accumulation of the pigment appears to be age-related since it was seen in all but one turtle over the age of sixteen examined in our study. Accumulation of lipofuscin or ceroid in senescent tissues in our study is consistent with other reports. Both pigments accumulate in non-replaceable aging cells such as nervous tissue and muscle (Christiansen and Gyrzbowski 1983, Pathy 1985). Lipofuscin has been observed in the corpus luteum of the skin, testis, adrenal cortex, and fat of aging humans (Anderson 1971), and ceroid has been noted in liver (Pathy 1985).

It appears that the tubular epithelium with accumulations of pigment are associated with failing nephrons. The tubules seemed to have a smaller lumen, and to be filled with large amounts of debris. If one or two cells in a tubule had pigment, the whole tubule had a different appearance from tubules with no pigment. It is not clear why lipofuscin was absent in the turtle heart muscle, because cardiac muscle is non-replaceable in humans. We could speculate that there could be at least two factors that might result in the absence of this pigment in reptilian myocardium. It would be absent if cardiac muscle tissue replaces itself in reptiles and we have no evidence that it does. It would be absent if these cells have a mechanism for excreting it and it is phagocytosed from the interstitial space. Christiansen (Pers. Comm.) has observed this pigment in the melanomacrophages of this species

The functional significance of lipofuscin is unknown, but Dorland (1988) states that it is the product of oxidation and polymerization of the membrane lipids of autophagocytosed organelles, so this suggests that it may be associated with old or dying

cells. It is clear from this study that accumulation of lipofuscin or ceroid is in some way associated with senescent cellsof the renal tubules.

It appears that several glomeruli in the older turtles have a decrease in the vascular to nonvascular tissue ratio. The change in this ratio could be due to a hypertrophy of the mesangial cells. It is possible that the increase in supporting tissue is masking arterioles, or that functional arterioles are shortening. Darmady et al. (1973) report that in some degenerating glomeruli the arterioles shorten and over time, the arterioles become so shortened that the afferent arteriole becomes directly continuous with the efferent arteriole.

The increase in glomerular connective tissue seen in our study is consistent with other studies. An increase in thickness of the glomerular arteriole basement membrane in humans was reported by Darmady et al. (1973) and Radke (1994). Anderson (1971) suggests that the increase in glomerular connective tissue may be scar tissue composed of deposits of basement membrane. Our slides stained for connective tissue were consistent with these results. The older kidneys appeared to have an increase in thickness of the basement membranes. These findings suggest glomerulosclerosis may be occurring and this has also been reported in aging human kidneys (Radke 1994). The oldest kidneys also appeared to have an increase in connective tissue throughout the kidney, though we were unable to quantify it. We did not test for the increase in interstitial collagen cross-linking observed by Darmady et al. (1973) and Radke (1994).

Electron microscopic studies provided further support for the light microscope findings. Glomeruli of the older turtles appeared to lack cellular detail. Very few podocyte nuclei were visible on the glomerular surface of the old turtles, but they were abundant on young glomeruli. The older glomeruli appeared to be simply masses of amorphous cells with no arteriolar structure visible. This was consistent with our observation at the light microscope level of an increase in mesangium.

Podocyte foot processes in old turtles were wider and disordered, and pedicels were disordered or completely absent. Where pedicels were still present on the foot processes, they were not neatly interdigitating with pedicels from neighboring foot processes as they were in young turtles, and they varied in length and width. It may be possible that these glomerular structures are changing in an effort to compensate for the loss of functional arterioles seen with the light microscope. A study by Heudes et al. (1994) suggested that human podocytes accumulate droplets of albumin and then hypertrophy. Some of the hypertrophied podocytes become detached from the basement membrane. It is possible that the changes seen in the spacing and regularity of the foot processes and pedicels may affect the glomerular filtration rate and differential permeability, and thus, kidney function.

Darmady (1973) reported that electron microscopic studies showed a hypertrophy of glomeruli in humans, and this change seemed to be evident in turtles. Glomeruli from young and old turtles examined at the same magnification by electron microscope appeared to be different in size. The glomeruli and glomerular structures such as podocyte cell bodies, foot processes, and pedicels from the older turtles seemed to be larger, and this parallels Darmady's (1973) studies on aging human kidneys.

Kidneys of turtles have not previously been examined by electron microscope Our light and electron microscope studies demonstrate that reptilian kidneys clearly differ from those of mammals. An electron micrograph of human kidney (Ross and Romrell 1985, Kessel 1979) shows very regular spacing of podocyte foot processes and pedicels. We observed that young and middle aged kinosternid turtles do have some order, but even with young turtles the foot processes and pedicels are less ordered (Figures 7A and 8A) than in humans.

Although several studies of mammalian kidneys report a loss in the number of glomeruli with age (Darmady et al. 1973, Goyal 1982, Moore 1931, Heudes et al. 1994),

no conclusive evidence suggests that this is the case in turtles. Christiansen and Gryzbowski (1993) state that the human kidney may lose up to one fifth its total volume. This could be an explanation for the lack of a decrease in the amount of glomeruli present per unit area. Perhaps there is a decrease in the total number of glomeruli, but it is not apparent because the total volume of the kidney has decreased. This could make it appear that the number of glomeruli does not decline when in fact the number is the same per unit area, but fewer total glomeruli are present in smaller old kidneys.

CONCLUSION

Histological examination of the kidneys of the Yellow mud turtle, *Kinosternon flavescens*, shows distinct senescence. An age-related pigment, lipofuscin or ceroid, is present in the old turtles and lacking in the youngest. Glomerular sclerosis occurs only in older turtles, as evidenced by the increase in connective tissue and in the decreased ratio of vascular to nonvascular tissue. Electron microscopic studies support the age-related renal decline seen by light microscope. Older turtles demonstrated a loss of glomerular detail and changes in both the foot processes and the pedicels of podocytes. Mud turtle renal senescence, therefore, parallels human renal senescnece in several ways.

To our knowledge, no other histological studies have found evidence for senescence in turtles. The turtles in this study were from a wild natural population, and they offer a picture of the aging process in turtles that are basically free from human influence. Turtles can be helpful in studying the evolution of senescence because they are similar to the early descendants of cotylosaurs, an ancestral reptile from which all reptiles and mammals evolved (Porter 1972). A common evolutionary origin of the senescence process suggests a common genetic cause. As with any malady, understanding the cause is basic to finding the cure.

This study provides a foundation for further work on chelonian renal senescence and physiology. We have no evidence as to how widespread the senescence process we observed in a Kinosternidae turtle is among other turtle families. It appears that *Kinosternon flavescens* is a reasonably short lived turtle and it would be interesting to know whether this process is paralleled in kidneys of long-lived turtles. The renal changes we observed should be associated with decreased renal function such as inulin or creatinine clearance. To our knowledge, such renal function tests have not been performed in a comparative study of young and senescent turtles.

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